PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P24534PC00	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/ZA 03/00092	International filing date *(day/month/year)* 15.07.2003	Priority date *(day/month/year)* 18.07.2002
International Patent Classification (IPC) or both national classification and IPC C12M1/26		
Applicant AGRICULTURAL RESEARCH COUNCIL et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand 23.01.2004	Date of completion of this report 06.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Saunders, T Telephone No. +31 70 340-4480

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/ZA 03/00092

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-12 as originally filed

Claims, Numbers

1-26 filed with telefax on 09.09.2004

Drawings, Sheets

1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-20,22,23
	No:	Claims	1,2,21,24-26
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-26
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents :

D1: US-A-5507133
D2: US-A-5994129
D3: WO-A-9015527
D4: WO-A-0170935
D5: WPI Accession Number 2000-431742 & ZA-A-9905408
D6: US-A-4358539

2. Novelty (Article 33(2) PCT)

2.1 D1 discloses (cf. Figure 1; claim 4) a device from which the subject-matter of claims 1 and 26 differs in that the profileration chamber is specifically anaerobic. The flexible bags used to form the inoculation and growth chambers are typically made from polyethylene, which would not be suitable for providing anaerobic proliferation conditions due to its relatively high oxygen permeability.

D1 is therefore not relevant to the novelty of claims 1-26.

2.2 D2 also discloses (cf. Figure 2; claim 1) a device from which the subject-matter of claims 1 and 26 differs in that the profileration chamber is specifically anaerobic

D2 is therefore not relevant to the novelty of claims 1-26.

2.3 D3 discloses (cf. claims 13, 26-28 and 31) a device comprising a proliferation chamber containing a tissue culture growth medium separated from an inoculation chamber containing a plant tissue culture by a partition which can be broken from the outside of the device without exposing either of the chambers to the external environment. The device appears to be both disposable and portable.

Although it is stated that the proliferation chamber includes a gas-permeable membrane, proliferation can be carried out anaerobically (cf. page 15, paragraph 2). For this to happen the proliferation chamber would clearly have to be anaerobic and

indeed D3 discloses (cf. page 15, paragraph 2) that the cellule used to culture microorganisms that live and grow anaerobically can be made from less permeable materials.

The growth medium is e.g. a legume inoculant culture and the inoculum comprises legume seeds (cf. pages 27-28).

The inoculum and uninoculated growth medium are stored and transported separate from each other towards a point of use and the inoculated seeds are dispensed from the container for sowing (cf. page 31, paragraph 3).

2.4 D4 discloses (cf. page 10, lines 15-24) a method from which the subject-matter of claim 21 differs in that proliferation specifically takes place under anaerobic conditions and is therefore not relevant to the novelty of claims 21-24.

2.5 The subject-matter of claims 1, 2, 21 and 24-26 is therefore not novel.

3. Inventive Step (Article 33(2) PCT)

3.1 Dependent claims 3-20, 22 and 23 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step.

3.2 D1 discloses the sterilisation by means of irradiation of a bag used as a portable cell, tissue and/or microorganism delivery apparatus prior to inoculation. A clamp between the proliferation and and inoculation chambers can be released in order to connect the insides of the chambers with each other - in an anaerobic device this would not compromise the anaerobiosis of either chamber.

D2 discloses a closed cell, tissue and/or microorganism delivery apparatus which is sterilisable as a unit.

D5 discloses the use of flexible infusion bags for profileration chambers.

D6 discloses the use of a rupturable septum for separating proliferation and inoculation chambers in a disposible subculturing device.

CLAIMS

1. A unitary disposable and portable cell, tissue and/or microorganism proliferation and delivery apparatus comprising at least one anaerobic proliferation chamber for containing a growth medium; at least one inoculation chamber for containing an inoculum; and means for separating the proliferation and inoculation chambers, the separating means being openable to connect the insides of the chambers to each other to inoculate the growth medium with the inoculum, to allow proliferation of the said cell, tissue and/or microorganism under anaerobic conditions, wherein the inoculum is provided in a form which is stable and viable beyond the normal life-span of a conventional culture in a closed container.

2. Apparatus according to claim 1, wherein the arrangement is such that the inoculum and growth medium are stored and transported separated from each other in the apparatus, until such time as a proliferated culture is to be applied, whereupon the growth medium is inoculated and proliferation allowed to take place, whereafter the proliferated culture is dispensed from the apparatus.

3. Apparatus according to any one of the preceding claims wherein the separating means and inside of the proliferation chamber are rendered sterile prior to inoculation.

4. Apparatus according to any one of the preceding claims wherein the inoculation chamber is also anaerobic.

5. Apparatus according to claim 4 which is provided with opening means for opening the separating means, without compromising the anaerobiosis of the inside of the chambers, the arrangement being such that the growth medium is inoculated and the microorganism proliferated anaerobically and aseptically.

6. Apparatus according to any one of the preceding claims which is totally enclosed and hermetically sealed.

7. Apparatus according to any one of the preceding claims wherein the chambers are connected to each other via a passage.

8. Apparatus according to claim 7 wherein the separating means is in the form of a septum.

9. Apparatus according to claim 8 wherein the opening means is in the form of a spike for piercing the septum.

10. Apparatus according to claim 9 wherein the inoculation chamber is defined by a vial-type container having a mouth which is connected to one end of the passage.

11. Apparatus according to claim 10 wherein the said septum covers the said mouth.

12. Apparatus according to claim 10 or 11 wherein the spike is mounted in the passage directed at the septum, and wherein the inoculation chamber is connected to the said one end of the passage via advancement means, the arrangement being further such that, in use, the inoculation chamber is advanced inwardly towards the spike, until the spike pierces the septum.

13. Apparatus according to any one of claims 8 to 12 wherein the vial-type container is flexible, the arrangement being such that, in use, the inoculation chamber is compressed after the septum has been opened to inoculate the growth medium.

14. Apparatus according to any one of claims 8 to 12 wherein the apparatus is provided with urging means for urging the inoculum into the proliferation chamber after the septum has been opened to inoculate the growth medium.

15. Apparatus according to any one of claims 8 to 12 wherein there is a pressure differentiation between the two chambers causing the inoculum to flow into the proliferation chamber after the septum has been opened to inoculate the growth medium.

16. Apparatus according to any one of the preceding means which is provided with a port for connecting to a dosing or application means.

17. Apparatus according to claim 16 wherein the arrangement is such that pressure, which builds up in the proliferation chamber during the anaerobic cultivation of the microorganism, urges the proliferated culture through the said port.

18. Apparatus according to any one of the preceding claims wherein the proliferation chamber is defined or provided by a flexible infusion bag type container.

19. Apparatus according to any one of claims 1 to 17 wherein the proliferation chamber is in the form of a "carboy"- type container.

20. Apparatus according to any one of the preceding claims which includes additional proliferation inoculation chambers connectable to the other chambers.

21. A method for the proliferation and delivery of cells, tissue cultures and/or microorganisms including the steps of :

- disposing an inoculum in an inoculation chamber;
- disposing a growth medium for the inoculum in an anaerobic proliferation chamber which is separated from the inoculation chamber by an openable separating means;
- storing and transporting the inoculum and uninoculated growth medium separated towards a point of use;
- opening the separating means to inoculate the growth medium;
- allowing the cells, tissue cultures and/or microorganisms to proliferate under anaerobic conditions in the proliferation chamber to form a proliferated culture; and
- dispensing the proliferated culture from the proliferation chamber.

22. A method according to claim 21 wherein the inoculation chamber is also anaerobic and wherein the steps of disposing, storing, transporting, inoculating, opening, and proliferation take place anaerobically.

23. A method according to claim 21 or 22 which includes the further step of controlling and/or adjusting proliferation conditions of the inoculated growth medium.

24. A method for the proliferation and delivery of cells, tissue cultures and/or microorganisms substantially as herein described with reference to the accompanying drawings.

25. A unitary cell, tissue and/or microorganism proliferation and delivery apparatus substantially as herein described and as illustrated in the accompanying drawings.

26. A unitary disposable and portable cell, tissue and/or microorganism proliferation and delivery apparatus comprising at least one anaerobic proliferation chamber for containing a growth medium; at least one inoculation chamber for containing an inoculum; and means for separating the proliferation and inoculation chambers, the separating means being openable to connect the insides of the chambers to each other to inoculate the growth medium with the inoculum, to allow

proliferation of the said cell, tissue and/or microorganism under anaerobic conditions, wherein the arrangement is such that the inoculum and growth medium are stored and transported separated from each other in the apparatus, until such time as a proliferated culture is to be applied, whereupon the growth medium is inoculated and proliferation allowed to take place, whereafter the proliferated culture is dispensed from the apparatus.